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July 1, 2004

Michael O. Leavitt, Administrator
US Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: Comments on the HPV test plan for the chemical 2-vinylpyridine

Dear Administrator Leavitt:

The following are comments on the test plan for the chemical 2-vinylpyridine (CAS# 1100-69-6) for the HPV program, submitted by Reilly Industries, Inc. (Reilly). These comments are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health and environmental protection organizations have a combined membership of more than ten million Americans.

Reilly proposes to do an OECD 421 screening protocol on this chemical, which will kill approximately 675 animals, and an OECD 203 study, which will kill approximately 40 fish.

The chemical is, to quote the test plan, "corrosive to tissues, flammable, and acutely toxic by oral and dermal routes...rats administered 2-Vinylpyridine by oral gavage developed clear irritation, cell proliferation and thickening of the tissues in the forestomach, the site of contact of the test material. No systemic effects were noted through histological analysis… " Chemicals that are classified as irritating will not likely cause systemic toxicity at doses which do not also cause significant local GI effects. All three cited repeat dose studies share this principle. Thus, the interpretation of any systemic effects that may be observed in proposed reproductive or developmental studies will be confounded by local effects due to the irritancy of the compound. Since it has been reported in the developmental toxicology literature that maternal stress may be related to developmental effects, it would e difficult to imply causation in the event of a positive result, since 2-vinylpyridine is so acutely toxic and corrosive. Additionally, the irritancy potential is such that testing would result in extreme suffering for the animals involved. Other public commenters have pointed out at other times that chemicals with such properties should not be subject to further testing in animals, and the EPA has accepted this principle in its consideration of other HPV test plans on similarly corrosive chemicals.

Moreover, any further information about other kinds of toxicity will not change the regulatory framework or protective regulations that already exist and govern the production and use of 2-vinylpyridine. Since the chemical is a human skin sensitizer, flammable, and corrosive, there are significant regulations in place regarding clean-up and personal protective procedures. Therefore, further animal testing will not result in additional protective measures being adopted. To see the specific guidance concerning this principle, please see *http://www.epa.gov/chemrtk/ceoltr2.htm.* To quote, "As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant."

We would also like to question the need for an OECD 203, as Reilly itself states in its test plan that "substantial toxicity to fish, aquatic invertebrates and algae is not expected." Since both TETRATOX and ECOSAR were used, there is no reason to conduct a live fish test. In the TETRATOX assay, the protozoan *Tetrahymena pyriformis* is used as a biomarker for acute lethality in fish (Schultz 1997). The biochemistry and physiology of *T. pyriformis* have been thoroughly investigated since the 1950s, and this assay has been used, in various forms, for aquatic toxicity testing since the 1970s (Sinks & Schultz 2001). In this test, a range-finding study followed by three replicate definitive tests is performed for each test substance. Each treatment replicate consists of a minimum of five different concentrations per substance tested; thus, at least 30 data points comprise each analysis. The current, standardized protocol is for a 40-hour static test, which provides for multigenerational exposure. Range-finding tests are also included to allow an accurate approximation of both the highest concentration with no observed effect on population growth and the lowest concentration with total inhibition of cell replication. Output measures from the TETRATOX assay are the 50 percent inhibitory growth concentration (IGC50, mmol/L) and the 95 percent fiducial interval. The current TETRATOX database includes more than 2,000 industrial organic chemicals, including over 800 aliphatic chemicals, 900 aromatic chemicals, 400 neutral narcotics, and 400 direct-acting electrophiles, among others (Schultz, personal communication). The TETRATOX protocol has now been standardized and has undergone a preliminary ring test (Larsen *et al.*1997). The German EPA is currently funding a second, more elaborate ring test, with the goal of establishing an OECD Test Guideline. In the interim, data generated by TETRATOX demonstrate a consistently high degree of concordance to data from *in vivo* acute studies in fish, which supports the use of this assay as a prospective replacement for toxicity studies in fish (Seward *et al.*2001). When combined with results from ECOSAR, there is simply no justification for further fish toxicity testing.

Thank you for your attention to this issue. We look forward to a prompt and favorable response to our concerns. We can be reached at 202-686-2210 ext. 335 or via email at *kstoick@pcrm.org.*

Sincerely,

Kristie Stoick, MPH
Research Analyst

Chad B. Sandusky, PhD
Director of Research

Larsen J, Schultz TW, Rasmussen L, Hoofman R & Pauli W. 1997. Progress in an ecotoxicological standard protocol with protozoa: results from a pilot ring test with *Tetrahymena pyriformis. Chemosphere,* 35:1023-1041.

Schultz TW. 1997. TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint: a surrogate for fish lethality. *Toxicol Meth* 7:289-309.

Sinks GD & Schultz TW. 2001. Correlation of *Tetrahymena* and *Pimephales* toxicity: evaluation of 100 additional compounds. *Environ Toxicol Chem* 20:917-921.

Seward JR, Sinks GD & Schultz TW. 2001. Reproducibility of toxicity across mode of toxic action in the *Tetrahymena* population growth impairment assay. *Aquat Toxicol* 53:33-47.